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A SUBMUCOSAL MECHANISM FOR CATECHOLAMINE-INDUCED INCREASES IN FLUID ABSORPTION IN RABBIT ILEUM *IN VITRO*

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SUMMARY

1. The effects of clonidine and dopamine on water movements across the mucosal and serosal surfaces of rabbit ileum have been investigated using a high-resolution method for monitoring water flows *in vitro*.

2. Theophylline (10 mM) and carbamyl choline (10 μM) caused a reduction in fluid inflow across the mucosal surface and a smaller decrease in fluid outflow across the serosal surface. Addition of the α_2-adrenergic agonist clonidine or dopamine fully reversed the theophylline, or carbamyl choline-induced decrease in mucosal inflow in a dose-related manner.

3. The effects of clonidine on mucosal inflow are blocked by the α_2-adrenergic antagonist, yohimbine. Yohimbine was much less effective than pimozide or *d*-butaclamol in blocking the effect of dopamine on mucosal inflow. These findings support the view that there are separate α_2-adrenergic and dopaminergic receptors.

4. The hydraulic conductance (L_p) of the serosal surface was measured directly from the change in serosal exit flow following addition of 2 mosmol kg^{-1} of polyethylene glycol (molecular mass 20000 Da) to the serosal bathing solution. Theophylline reduced the L_p by 35%. Clonidine (1 μM) added to theophylline-treated tissues increased the L_p by 66%. This effect was prevented by yohimbine (1 μM).

5. The effects of theophylline, clonidine and dopamine on the permeability of the mucosal and serosal surfaces of the tissue to [^{3}H]mannitol were measured. These showed that theophylline increased the rate of labelled mannitol loss across the mucosal surface but reduced the mannitol permeability across the serosal surface. This latter effect was reversed by clonidine and dopamine.

6. Changes in transepithelial electrical potential difference (PD), short-circuit current and resistance were monitored. Theophylline caused a rapid increase in PD and short-circuit current and a slower increase in resistance. Clonidine (5 μM) reversed the effects on PD and resistance but was without significant effect on short-circuit current. The results suggest that a major component of secretagogue-induced reduction in fluid transport *in vitro* is due to mechanical changes in the submucosa, probably induced by modulation of neurotransmitter release within the tissue.

INTRODUCTION

Electrolyte absorption in rabbit ileum is stimulated by adrenergic (Field & McColl, 1973) and dopaminergic agonists *in vitro* (Donowitz, Elta, Battisti, Fogel & Schwartz, 1983). Similarly fluid and electrolyte absorption are stimulated by adrenergic agonists, in rat (Hubel, 1976) and cat *in vivo* (Sjövall, Redfors, Hallbäck, Eklund, Jodal & Lundgren, 1983). The adrenergic action *in vitro* is mediated by α_2-adrenoreceptors (Chang, Field & Miller, 1982) and the dopamine-dependent stimulation of electrolyte transport, via dopaminergic receptors (Wahawisan, Gaginella & Wallace, 1985) although Donowitz *et al.* (1983) claim that dopamine acts via α-adrenergic receptors.

It has been reported that adrenergic agents decrease cyclic AMP levels in rabbit ileal mucosa exposed to prostaglandins, or cholera toxin (Field, Sheerin, Henderson & Smith, 1975). However, Nakaki, Nakadate, Yamamoto & Kato (1982*a*) found no clonidine-dependent reduction of the augmented levels of cyclic AMP induced by exposure to prostaglandin E_1 (PGE_1) or VIP in isolated rat enterocytes. Dopamine does not affect tissue levels of cyclic AMP, but decreases in total tissue calcium and ^{45}Ca uptake were observed consistent with a reduction in intracellular Ca^{2+} (Donowitz, Casolito, Battisi, Fogel & Sharp, 1982).

It is thought that both adrenergic and dopaminergic agonists act directly on the enterocytes for the following reasons: (a) binding studies show that the α_2-adrenergic agonist clonidine binds to isolated enterocyte membranes (Nakaki, Nakadate, Yamamoto & Kato, 1983), (b) adrenergic and dopaminergic agonists are said to have direct effects on second messenger concentrations within the mucosa (Field *et al.* 1975; Donowitz *et al.* 1982), (c) clonidine decreases short-circuit current *in vitro* and increases net Na^+ and Cl^- absorption in rabbit ileum (Chang *et al.* 1982) and (d) clonidine enhances the rate of fluid absorption in rat intestine *in vivo* exposed to dibutyryl cyclic AMP (Wahawisan *et al.* 1985) and in cat intestine fluid absorption is increased in response to electrical stimulation of the adrenergic splanchnic nerve (Sjövall *et al.* 1983).

It is assumed that the electrical effects and asymmetric transepithelial transport can only arise from direct stimulation of enterocytes. However, the simple view that adrenergic or dopaminergic effects on intestinal electrolyte and fluid transport are due to a reversal of the changes in enterocyte second messenger concentrations and hence to a reversal in the secretagogue-induced changes in cell membrane ion conductances is not fully in accord with the following published data. (i) There is no clonidine-dependent reduction of the augmented levels of cyclic AMP induced by PGE_1 or VIP in isolated rat enterocytes (Nakaki *et al.* 1982*a*, *b*). (ii) Noradrenaline does not reverse the increase in short-circuit current induced by cyclic AMP in rabbit intestine *in vitro* (Field & McColl, 1973). (iii) Although cholinergic agonist-induced secretion in rabbit ileum *in vitro* is not mediated by changes in cyclic AMP (Bolton & Field, 1977), electrolyte secretion induced by cholinergic agonists is reversed by intrinsic release of adrenergic agonists. This effect is blocked by the α-adrenergic antagonists phenoxybenzamine and phenotolamine (Tapper, Powell & Morris, 1978).

The inconclusive evidence that changes in epithelial cells are the major regulators

of intestinal fluid movement *in vitro* led us to investigate whether changes in smooth muscle tone in the submucosa could play a significant role in the control of net fluid absorption. α_2-Adrenergic and dopaminergic receptors exist on presynaptic terminals of cholinergic myenteric plexus neurones. α_2-Adrenergic agonist binding to these receptors inhibits stimulated acetylcholine release (Paton & Vizi, 1969; Drew, 1978; Kilbinger & Wessler, 1979). Also dopaminergic agonists inhibit acetylcholine release from guinea-pig myenteric plexus (Vizi, Ronai & Knoll, 1974; Gorich, Weihrauch & Kilbinger, 1982).

A possible mode by which adrenergic and dopaminergic agonists increase intestinal fluid and electrolyte transport is by relaxing smooth muscle tone. The submucosal tissues act as the major resistance to transepithelial ion and water movements in rabbit ileum *in vitro* (Naftalin & Tripathi, 1985, 1986) and rat intestine (Fromm, Schulzke & Hegel, 1985). Opiate-induced relaxation of submucosal tone can increase fluid and ionic conductance without affecting the secretagogue-induced increase in short-circuit current (Ahsan, Ilundain, Naftalin, Sandhu & Smith, 1987).

Therefore we examined the effects of theophylline, carbamyl choline, clonidine and dopamine on hydraulic and electrical conductances of the submucosal tissue and the mucosal and serosal permeability to the extracellular marker [^{3}H]mannitol.

METHODS

Animals

Male New Zealand White rabbits weighing between 2 and 3 kg were killed with intravenous sodium pentobarbitone and the ileum rapidly removed and its contents washed free with Krebs–Ringer solution (mM: NaCl, 137; KCl, 5·7; $CaCl_2$, 1·8; $MgSO_4$, 1; Na_2HPO_4, 0·4; $NaHCO_3$, 11·7) to which D-glucose (11 mM) was added. The ileum was dissected free of mesentery and longitudinal muscle and then mounted in an Ussing-type chamber which contained approximately 20 ml solution in each hemichamber. The serosal surface was supported by a fine mesh grid and the mucosal solution only was gassed with 95% O_2:5% CO_2. Previous studies have shown that such conditions are sufficient to maintain adequate oxygenation and pH control of the ungassed serosal compartment (Naftalin & Tripathi, 1986).

Water flows

The method of monitoring intestinal fluid movement has been fully described (Naftalin & Tripathi, 1985, 1986). Flow of water from the tissue into the serosal solution, J_s, was monitored using a capacitance probe (Wayne Kerr, Bognor Regis), which measures the width of an air gap between the probe surface and the meniscus of the serosal bathing solution. Absorptive flow across the tissue increases the amount of fluid in the serosal bathing solution, so the air gap between the surface of the serosal solution and the capacitance probe decreases. The output from the capacitance probe was recorded continuously on a chart recorder. The exposed serosal surface area was 10 cm^2; evaporative losses were constant, so the flow, J_s (μl cm^{-2} h^{-1}), was obtained from the slope of the output signal plotted on a chart recorder (JJ Instruments Ltd, Southampton).

The change in tissue volume, J_t, was also monitored continuously with an optical lever. This consisted of a lightweight mirror pivoted at its lower edge. The upper edge rested directly against the mucosal surface of the tissue in the bathing solution. Expansion of the tissue caused a forward rotation of the mirror, which changed the angle of reflection of a low-power laser beam (Spectraphysics Ltd). The beam displacement was monitored using a pair of photodiodes and the signal displayed on a second channel of the chart recorder. The signal was calibrated for tissue expansion using a micrometer-controlled displacement of the optical lever. Whilst small changes in the mirror displacement could be due to local displacement in the immediate vicinity of the mirror these effects cannot account for the prolonged changes in flow observed or for the reproducibility of the flows with different agents.

Care was taken to ensure that the tube delivering the gas stream to the mucosal solution terminated above the upper edge of the mirror. In this way mirror vibration by the gas stream was avoided. Flow across the mucosal surface of the tissue, J_m, was equal to the algebraic sum of J_s and J_t, the tissue volume change (cm serosal area^{-2}).

This approach to measuring intestinal fluid movements has several advantages over measurement of ion movements using tracer methods: net changes in flow are measured directly; the resolution is more than 20-fold above that used to estimate ion fluxes, hence changes in flow within 1 min after altering the experimental conditions are easily detectable; a second advantage is that flows are simultaneously measured across both the mucosal and serosal surfaces. The independent changes observed in flow across the two surfaces following treatment with drugs gives direct information concerning the separate determinants of flow across the mucosal and submucosal surfaces of the tissue.

On the occasions when the mucosal solution was not gassed adequately rhythmic contractions of the circular muscle and muscularis mucosa developed. This was observed as a sinusoidal movement of the mirror and the meniscus of the serosal solution with a periodicity of 30–60 s. This spontaneous contraction is not normally seen in rabbit ileum, even after prolonged incubation. On the few occasions it did occur the experiment was terminated.

Experimental protocol

After mounting the tissue and adjusting the probes, which takes between 10 and 15 min, the tissue flows were monitored for 15–30 min, the solution temperature equilibrated to 35–36 °C and maintained by thermostatic control using a heating water circulator (Haake model E3). Absorptive fluid movement increased rapidly to reach a quasi-steady-state (J_m was 10–25 μl cm^{-2} h^{-1}) and flow across the serosal border, J_s, rose slowly from approximately 5 μl cm^{-2} h^{-1} to between 10 and 15 μl cm^{-2} h^{-1}. Thus in control tissue there was a steady rate of tissue swelling at a rate of 10–15 μl cm^{-2} h^{-1}. This oedema formation in absorbing tissue has been noted previously (Parsons & Wingate, 1961; Van Os, Weidner & Wright, 1979) and there is a highly significant correlation between tissue wet:dry weight ratios and the amount of osmotic swelling or shrinkage monitored by the optical lever (R. J. Naftalin & S. Tripathi, unpublished results).

Because recovery of flow after exposure to high concentrations of active drugs was slow and the tissue response was reduced after prolonged incubation (for more than 4 h) the optimal way of obtaining a graded response was to add the drugs in increasing concentrations at intervals of 15–20 min. This allowed the tissue to reach a new steady-state rate.

Measurement of the osmotic hydraulic conductance of the serosal surface

In the absence of a colloid osmotic pressure gradient net fluid flow across the serosal surface of the tissue is generated entirely by interstitial pressure. It has long been recognized that intestinal fluid movements are limited by the hydraulic permeability of the submucosal tissues (Smyth & Taylor, 1957; Parsons & Wingate, 1961). However, the extent of this barrier has only recently been quantified. The serosal border of rabbit and rat intestine behaves as a membrane with low conductance ($L_p \approx 5 \times 10^{-8}$ cm s^{-1} cmH_2O^{-1}) and homogeneous porosity (mean pore radius $\approx$ 6·5 nm) (Naftalin & Tripathi, 1985). Fromm *et al.* (1985) deduced from electrical resistance parameters in rat intestine that the submucosal tissues constitute the major fraction of the electrical and hydraulic resistance of the tissue *in vitro*. The hydraulic conductance of the serosal surface, L_p (cm s^{-1} cmH_2O^{-1}), was determined as follows: addition of impermeant macromolecules to the serosal bathing solution induced osmotic flow across the submucosal layers (Naftalin & Tripathi, 1985). The L_p was estimated from the change in J_s mosmol^{-1} according to the following relationship:

$$\delta J_s = L_p RT \delta C,$$

where RT is the product of the gas constant and T = K, hence RT at 36 °C $\approx$ 24 cmH_2O mosmol^{-1}; δC is the osmolar concentration of polyethylene glycol added to the serosal bathing Ringer solution, obtained from vapour pressure readings using a Wescor vapour pressure osmometer. The concentrations of polyethylene glycol (molecular mass 20000 and 90000 Da) were adjusted to give osmotic activities of 2 and 1 mosmol kg^{-1} respectively. Two sizes of polyethylene glycol were used to ensure that no error was introduced because of permeation of smaller macromolecules into the tissue. The following criteria were used to exclude significant macromolecular permeation across the serosal surface. (a) A step change in outflow after addition

of polyethylene glycol to the serosal solution followed by a continuous trace at a stationary rate for 5–10 min. (b) Following removal of the polyethylene glycol from the serosal solution the exit rate returns to control levels and does not fall significantly below control flows prior to addition of polyethylene glycol.

Determination of interstitial pressure, P_i, and tissue distensibility, K

Since flow across the submucosa is passive, net fluid outflow from the submucosa to serosal bathing solution, J_s, in the absence of a colloid osmotic pressure gradient across the serosal surface, is determined solely by the interstitial pressure, P_i, and L_p of the serosal surface, i.e.

$$J_s = L_p P_i \quad \text{or} \quad P_i = J_s/L_p.$$

The interstitial pressure, P_i, is inversely related to the volume elasticity, K (distensibility, or active muscle tone), of the submucosa and directly to the fractional distension, $\delta V/V_0$ of the submucosa relative to the resting level, where V_0 is the resting volume of the submucosa, i.e.

$$P_i = \frac{\delta V}{(K V_0)} \quad \text{and} \quad K = \frac{\delta V}{(P_i V_0)}.$$

It is possible to estimate the value of tissue distensibility, K, provided the fractional increase in interstitial volume $\delta V/V_0$ is known or assumed. The extracellular volume of the tissue estimated from the mannitol space was $\approx 73\ \mu l/cm^2$ (see Experimental protocol). In this series, net tissue expansion was approximately $5\ \mu l\ cm^{-2}\ h^{-1}$. Thus, after 90 min, the tissue expanded by approximately 10%. The bulk of this expansion is into the submucosa. Submucosal oedema is a recognized consequence of fluid absorption in *in vitro* intestinal preparations (Parsons & Wingate, 1961).

Given a 10% expansion in interstitial volume, the coefficient of volume distensibility, K, in control tissue (K_{con}) may be estimated from

$$J_s/L_p = P_i,$$

and

$$\frac{V}{V_0 P_i} = K_{con} \approx \frac{7{\cdot}3}{73 \times 42}\ cmH_2O^{-1} \approx 2{\cdot}4 \times 10^{-3}\ cmH_2O^{-1}.$$

Provided no large-scale change of tissue volume occurs following application of drugs, it is possible to estimate their effect on distensibility from the L_p determination and the flow J_s measured in the absence of a colloid osmotic gradient.

Measurement of steady-state retention volume of [^3H]mannitol within the tissue and its fractional exit across the mucosal surface

Transepithelial mannitol flows were measured in Ussing-type chambers which have been described previously (Naftalin & Holman, 1974). Each chamber device consists of six ports. In this series of experiments, 5 ml of solution was present on both sides. The temperature of the solutions was maintained at 35–36 °C with a Churchill heating water circulator which pumped water through an integral water jacket within each device. Oxygenation and vigorous stirring of the bathing solutions were maintained by a gas lift mechanism which bubbled 95% O_2:5% CO_2.

A sheet of ileum stripped of serosa and outer longitudinal muscle layers was mounted across all six chambers in each device so that neighbouring ports contained adjacent pieces of epithelium. The protocol used to monitor the effect of drugs on the tissue permeabilities to mannitol was as follows: the tissue was pre-incubated for a 30 min period at 35–36 °C in Krebs–Ringer solution; clonidine was added to the bathing solution when required during this period. ^3H-labelled mannitol was then added to the serosal bathing solution. Theophylline was also added at this time. After a single 30 min incubation period the mucosal and serosal bathing solutions were sampled and bathing solutions were then removed and after washing with Krebs–Ringer solution at 5 °C to remove surface adherent radioisotope, the remaining isotope was extracted from the tissue for 12–14 h, in 0·4% Triton X-100. The flux from serosal to mucosal side, J_{sm}, was obtained as follows:

$$J_{sm} = \frac{(C_{t1} - C_{t0})}{(H_{t0})} \frac{c}{A\,(t_1 - t_0)},$$

where H and C represent the activity in c.p.m. ml^{-1} in the 'hot' and 'cold' sides respectively.

Subscripted ts indicate the sampling time; (t_1-t_0) is the time interval between the two samples, c is the marker concentration (μmol cm^{-3}) in the 'hot' side and A is the exposed tissue area (1·7 cm^2).

Measurement of the amount of labelled extracellular marker (mannitol) within the tissue in conjunction with the steady-state rate at which the marker passes across the tissue permits three other system variables to be estimated in addition to transepithelial flux. (a) Steady-state retention volume (SSRV) was defined as the volume of exposed tissue (μl cm^{-2}) filled by the isotope and estimated as follows:

$$\text{SSRV} = \frac{T_i}{H}\frac{1}{A},$$

where T_i is the total extractable radioactivity within the tissue, H is the c.p.m. ml^{-1} of 'hot'-side solution and A is the exposed area of tissue. (b) Fractional exit (FE) (mucosal) is defined as the proportion of isotope within the tissue which crosses into the 'cold'-side solution during transepithelial serosal–mucosal flux. Given steady-state flux conditions, fractional exit of mannitol into the mucosal solution is related directly to the permeability of the mucosal boundary. FE is expressed as a fraction of activity lost per hour with reference to counts within the tissue, i.e.

$$(\text{FE})\,\text{h}^{-1} = \frac{\text{c.p.m. (lost to mucosal solution)}}{\text{c.p.m. (lost to mucosal solution)} + \text{c.p.m. (accumulated in submucosa)} \times 2}.$$

The factor 2 is required to convert the half-hour collection fraction to fractional loss per hour. Mannitol is restricted to the extracellular compartments of epithelial tissues (Smulders & Wright, 1971; Biber, Aceves & Mandel, 1972). However, it does cross the mucosal surface of rabbit ileum via paracellular shunt channels (Naftalin & Tripathi, 1985, 1986), thus the exit rate of mannitol is a measure of the permeability of extracellular channels and the steady-state retention volume of labelled mannitol monitored is a measure of the relative permeability of the mucosal and serosal surface of the tissue to mannitol. (c) Serosal permeability to mannitol, P_s, can be shown during steady-state serosal–mucosal flux of mannitol, where the steady-state retention volume (SSRV) is equivalent to

$$\text{SSRV} = V_e R_m/(R_m + R_s),$$

where V_e is total extracellular fluid volume = 73 μl cm^{-2} (serosal area) determined by experiments in which the tissue was equilibrated with label from both sides simultaneously; R_m and R_s are the resistances to flow of mannitol across the extracellular pathways of the mucosal and serosal surfaces (where $R_m = 1/P_m$ and $R_s = 1/P_s$; P (cm h^{-1}) is the permeability of the barriers to mannitol). Thus, the closer the steady-state retention volume approaches the extracellular volume, i.e. 73 μl cm^{-2} during serosal to mucosal flow of mannitol, the greater is the ratio R_m/R_s.

Since the mucosal fractional exit rate is directly related to P_m, determination of both FE (mucosal) and SSRV for mannitol permits P_s to mannitol to be estimated. Thus,

$$P_s = \text{FE}/(V_e/\text{SSRV} - 1).$$

Potential difference, short-circuit current and tissue resistance measurements

All electrical measurements, namely transepithelial potential difference, short-circuit current (corrected for solution resistance and electrode offsets) and tissue resistance determined by the voltage change induced by a short current pulse, were made using a microprocessor-based voltage clamp device (Naftalin & Smith, 1984). The device also times, measures, checks, stores, performs the statistical processing and displays in graphical, or numerical form, up to twelve independent parallel records.

Materials

Theophylline and all salts were obtained from Sigma, Poole, Dorset. Clonidine was obtained from Boehringer Ingelheim Ltd, Bracknell, Bucks; prazosin from Pfizer Ltd, Sandwich, Kent; *cis*- and *trans*-flupenthixol from Lundbeck Ltd, Luton, Beds; pimozide from Janssen Pharmaceuticals, Marlow, Bucks; yohimbine, isoprenaline, propanolol, dopamine and carbamyl choline from Sigma Chemicals Ltd, Poole, Dorset.

Polyethylene glycols, molecular masses 20000 and 90000 Da, were obtained from BDH, Poole, Dorset.

Radioisotopes

^{3}H-labelled mannitol from New England Nuclear (F.R.G.).

Radioactive counting

All radioisotopes were counted by β-emission in a Packard 3320 Tri-Carb liquid scintillation spectrometer. The scintillation counting fluid was composed of 2·5 g diphenyloxazole (PPO), 500 cm^3 toluene and 500 cm^3 Synperonic-X (detergent). This fluid was designed to accept a large aqueous sample (up to 20% by volume) at a low cost.

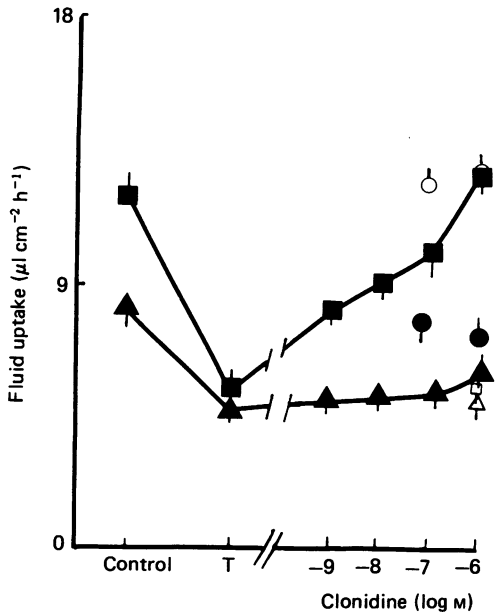

Fig. 1. Effects of the theophylline, T (10 mM), and clonidine on fluid uptake across the mucosal surface, $J_m \pm$ standard error of the mean (■), and serosal surface, $J_s \pm$ standard error of the mean (▲) ($n = 5$). Effects of yohimbine (1 μM) on J_m and J_s, □ and △ respectively, are also shown ($n = 3$). (IC_{50} for clonidine reversal of theophylline-dependent reduction in $J_m \approx 5$ nM.) The effects of clonidine alone on control J_m and J_s are shown in ○ and ● respectively ($n = 3$).

RESULTS

Effects of theophylline and clonidine on water movements across the mucosal and serosal surfaces

In Fig. 1 the effects of theophylline (10 mM) and varying concentrations of clonidine in the presence of theophylline (10 mM) on the fluid movements across the mucosal and serosal surfaces of the tissue are shown. Theophylline reduced steady-state net fluid flow across the mucosal surface from 13 to 6·5 μl cm^{-2} h^{-1} ($P < 0{\cdot}001$) and from 8·65 to 6·03 μl cm^{-2} h^{-1} ($P < 0{\cdot}01$) across the serosal surface simultaneously. Increasing concentrations of clonidine added to the mucosal and serosal bathing solutions caused a dose-related increase in J_m (concentration giving 50% maximum inhibition, $IC_{50} \approx 5$ nM); full recovery to the control influx was observed at a clonidine concentration of 1 μM. The changes in J_m induced by clonidine were fully developed within 1–2 min following application of the drug. In this series there was a small increase in J_s from 6·03 to 7·1 μl cm^{-2} h^{-1} when clonidine was raised to 1 μM;

at lower concentrations no significant increases in J_s were observed. The effects of clonidine on J_m were reversed by yohimbine (1 μM).

Clonidine in the absence of theophylline was without significant effect on flow across the mucosal border, J_m, or on flow across the serosal border, J_s, in control tissue, although at high concentrations (> 10 μM) it was sometimes observed to decrease J_m.

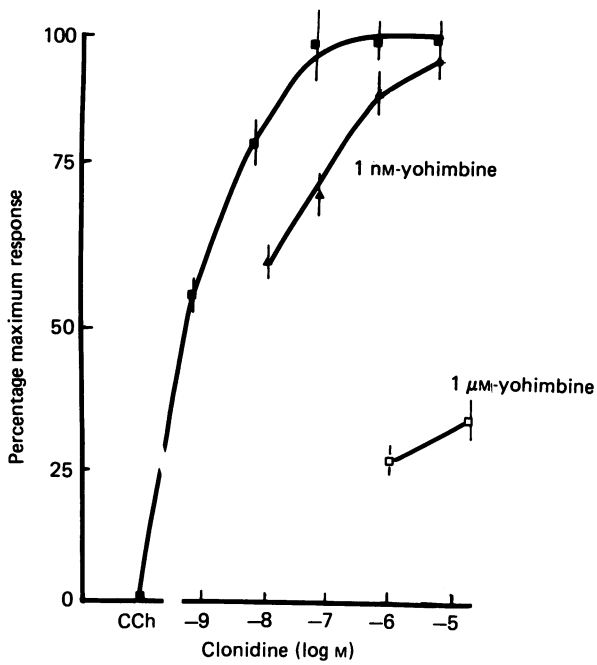

Fig. 2. The dose–response to clonidine following inhibition of J_m by carbamyl choline, CCh (10 μM), is shown ($n = 8$). The ordinate shows the percentage of the maximal effect on J_m induced by clonidine after exposure to carbamyl choline. The effects of 1 nM-yohimbine ($n = 3$) are shown by ▲ and 1 μM-yohimbine by □ ($n = 3$). In tissues treated with carbamyl choline alone (■) inflow, J_m, was $4{\cdot}9 \pm 0{\cdot}3$ μl cm^{-2} h^{-1} ($n = 8$); with yohimbine (1 nM) J_m fell to $3{\cdot}5 \pm 0{\cdot}5$ μl cm^{-2} h^{-1} ($n = 3$) and with yohimbine (1 μM) J_m fell to $3{\cdot}0 \pm 0{\cdot}5$ μl cm^{-2} h^{-1} ($n = 3$).

The effect of clonidine on fluid movements after exposure to carbamyl choline (10 μM)

In the presence of guanethidine hydrochloride (10 μM), which was added to prevent effects of release of intrinsic catecholamines (Tapper, Bloom & Lewand, 1981), carbamyl choline (10 μM) induced a rapid decrease in mucosal inflow, J_m, from $8{\cdot}6 \pm 0{\cdot}4$ to $4{\cdot}9 \pm 0{\cdot}3$ μl cm^{-2} h^{-1} ($n = 8$) ($P < 0{\cdot}01$). Addition of clonidine caused a dose-related increase in J_m ($IC_{50} = 5$ nM) but no significant change in J_s. Clonidine caused a full recovery in J_m. The lower control absorption in this series compared to that found in the series of experiments with theophylline may be due to reduced release of endogenous catecholamines from the intrinsic neural plexuses within the wall of the tissue, but this point was not specifically tested.

The percentage recovery of normalized values of J_m in the presence of carbamyl

choline (10 μM) with increasing concentrations of clonidine is shown in Fig. 2. In the presence of the α_2-adrenergic antagonist yohimbine (1 nM) there was a shift to the right in the response of J_m to the effect of clonidine ($P < 0.05$). A further rightward shift was observed when 1 μM-yohimbine ($P < 0.001$) was present; however, in this case full recovery to control values was not observed when carbamyl choline

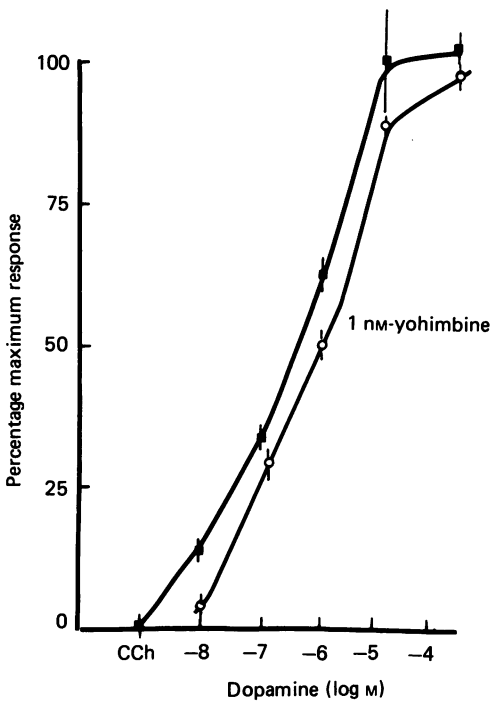

Fig. 3. The response to dopamine of percentage recovery of mucosal inflow following inhibition of J_m by carbamyl choline, CCh (10 μM), is shown (■, $n = 5$). Inhibition constant (K_i) for dopamine-dependent reversal of the carbamyl choline-induced effect is 0·5 μM. The ordinate shows the percentage of the maximal effect on J_m induced by dopamine after exposure to carbamyl choline. The effect of yohimbine (1 nM) in addition to dopamine is shown (○, $n = 3$). Carbamyl choline (10 μM) induced a rapid decrease in mucosal inflow, J_m, from $9{\cdot}1 \pm 0{\cdot}5$ to $4{\cdot}5 \pm 0{\cdot}3$ μl cm^{-2} h^{-1} ($n = 5$) ($P < 0{\cdot}01$). In the presence of yohimbine (1 nM) and carbamyl choline (10 μM) J_m was decreased from $8{\cdot}8 \pm 0{\cdot}5$ to $3{\cdot}5 \pm 0{\cdot}5$ μl cm^{-2} h^{-1} ($n = 3$).

concentration was raised, even to 1 mM (not shown). Yohimbine, even in the absence of clonidine and presence of guanethidine, had a small inhibitory effect on J_m, which augmented the decrease in the inflow observed in the presence of carbamyl choline (10 μM). For this reason the percentage recovery of flow was plotted as a function of clonidine, or dopamine concentration (Figs 2 and 3), rather than the absolute response. However, the extent of the recovery response in the presence of clonidine was approximately the same as that shown in Figs 1 and 4. These findings apparently suggest that the yohimbine did not behave as a competitive inhibitor at

higher concentrations. This uncompetitive action of yohimbine could be due to a non-linear tissue response, rather than to an aberrant pharmacological response.

The results confirmed the findings of Chang *et al.* (1982) that recovery in transmucosal flux following inhibition of absorption is increased by α_2-adrenergic agonists and inhibited by α_2-adrenergic antagonists.

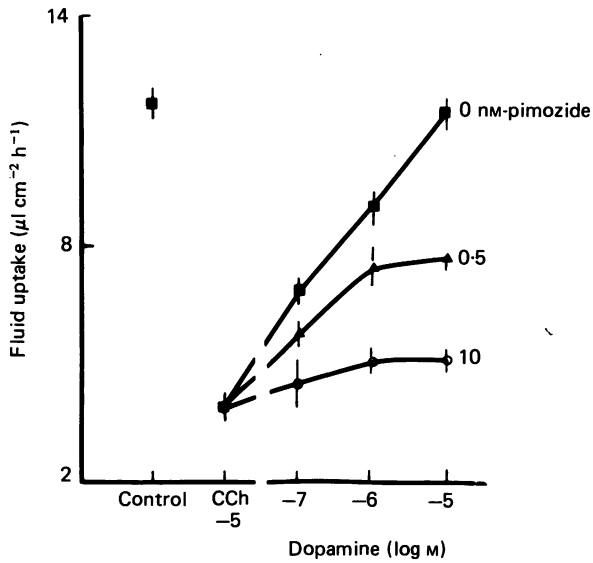

Fig. 4. Effect of carbamyl choline, CCh (10 μM), and dopamine (■) on J_m ($n = 5$) (μl cm^{-2} h^{-1}), alone and in the presence of 0·5 nM and 10 nM-pimozide (▲, $n = 3$) (○, $n = 3$).

Effects of dopamine and dopamine antagonists on fluid movements across the mucosal and serosal surfaces

Dopamine, like clonidine, increased fluid movement across the mucosal surface of rabbit small intestine which had previously been exposed to carbamyl choline (10 μM) and guanethidine (10 μM) (Fig. 3). A further resemblance to the action of clonidine is that it had no systematic dose-related effect on flow across the serosal surface, J_s. The threshold response of the mucosal inflow, J_m, to dopamine was observed at a dopamine concentration of 0·1 μM ($IC_{50} \approx 1$ μM). The decrease in mucosal inflow induced by carbamyl choline was fully reversed by 10 μM-dopamine. Yohimbine (1 nM) caused a smaller rightward shift in the response of J_m to dopamine than was found with clonidine (Fig. 2). This effect of yohimbine was considerably less than that observed with the D_2 dopamine antagonist pimozide (Fig. 4) and the mixed D_1 and D_2 antagonist *d*-butaclamol (1 nM) which completely prevented the effect of dopamine (1 μM) ($P < 0{\cdot}01$, $n = 3$). The inactive isomer *l*-butaclamol was less efficacious than the active isomer *d*-butaclamol. Neither *cis-* nor *trans-*flupenthixol were effective in reversing the effects of dopamine on fluid inflow. The results indicate that dopamine was acting on fluid absorption via a D_2 receptor.

Effects of clonidine, yohimbine and theophylline on the hydraulic conductance of the serosal surface

The hydraulic conductance of the serosal surface was determined as described in Methods. The changes observed in serosal outflows following addition of polyethylene glycol and the derived values of hydraulic conductance, L_p, are shown in Table 1.

TABLE 1. Hydraulic conductance of the serosal surface

* Test *vs.* control; † test *vs.* theophylline (*t* test). One asterisk or dagger symbol, $P < 0{\cdot}05$; two symbols, $P < 0{\cdot}01$.

Effects of addition of 2 mosmol kg^{-1} of polyethylene glycol (molecular mass 20000 Da) to the serosal solution on serosal outflow, J_s.

Value of control $L_p = 5{\cdot}7 \times 10^{-8}$ cm s^{-1} cmH_2O^{-1} and control $K = 2{\cdot}4 \times 10^{-3}$ cmH_2O^{-1}.

Condition	δJ_s (μl cm^{-2} h^{-1})	n	L_p ($\times 10^8$ cm s^{-1} cmH_2O^{-1})	K ($\times 10^3$ cmH_2O^{-1})
Control	10·7 ± 1·7	7	5·7 ± 1·0	2·4 ± 0·5
Theophylline (10 mM)	6·9 ± 0·8*	6	3·7 ± 0·5*	2·1 ± 0·3
Clonidine (1 μM)	12·3 ± 1·2	3	6·6 ± 0·7	2·9 ± 0·4
Clonidine (1 μM) + theophylline	11·4 ± 0·9††	7	6·3 ± 0·5††	3·1 ± 0·3†
Clonidine + yohimbine (1 μM) + theophylline	7·5 ± 0·5**	5	4·0 ± 0·2**	2·4 ± 0·2

Following exposure of the tissue to theophylline (10 mM), the increase in the rate of fluid outflow, δJ_s, elicited by a colloid osmotic pressure gradient decreased significantly. This indicates that the hydraulic conductance of the serosal border was decreased by theophylline from 5·7 to $3{\cdot}7 \times 10^{-8}$ cm s^{-1} cmH_2O^{-1} ($P < 0{\cdot}05$). There is a large increase in the rate of fluid exit elicited by the colloid osmotic pressure gradient when clonidine is added to tissue exposed previously to theophylline. The large clonidine-dependent increase in osmotic pressure-generated flux occurs despite the absence of any large change in serosal outflow, J_s, following addition of clonidine (1 μM) to tissues exposed to theophylline (Fig. 1). This effect of clonidine is prevented by yohimbine (1 nM) and hence indicates that the drug acts via α_2-adrenoreceptors. This paradoxical behaviour indicates that clonidine both decreased interstitial pressure and increased submucosal hydraulic conductance of tissue exposed to theophylline (see Discussion). Clonidine has no significant effect on the hydraulic conductance of control tissues.

Steady-state retention of [^{3}H]mannitol and fractional efflux of mannitol across the mucosal surface

The fractional exit of [^{3}H]mannitol across the mucosal surface was increased from 0·18 to 0·29 h^{-1} following exposure to theophylline (10 mM), indicating an increased paracellular permeation by the solute (Table 2). Additionally, theophylline decreased (i) the steady-state retention volume of mannitol within the tissue during transepithelial serosal–mucosal flux from 25 to 14·8 μl cm^{-2}, and (ii) the estimated mannitol permeability of the serosal surface.

The effect of theophylline on the fractional exit rate of mannitol across the mucosal surface was best observed in the period immediately after addition following a period of pre-incubation without theophylline. If theophylline was added in the pre-incubation period, the 'squirted' exit of mannitol across the mucosal surface was attenuated.

TABLE 2. Steady-state retention of [^{3}H]mannitol and fractional mucosal exit of mannitol and estimated serosal permeability

Condition	Steady-state retention (μl cm^{-2})	n	Fractional mucosal exit (h^{-1})	n	Serosal permeability (h^{-1})
Control	24·96 ± 0·92	67	0·18 ± 0·01	70	0·09 ± 0·01
Theophylline (10 mM)	14·77 ± 0·93***	48	0·29 ± 0·02***	81	0·07 ± 0·01*
Noradrenaline (10 μM)	22·15 ± 2·2	5	0·23 ± 0·01	5	0·10 ± 0·01
Clonidine (1 μM)	21·3 ± 1·3	5	0·26 ± 0·02*	5	0·11 ± 0·01
Theophylline + noradrenaline (10 μM)	21·33 ± 1·71†††	9	0·30 ± 0·05*	7	0·12 ± 0·02††
Theophylline + noradrenaline + propanolol (10 μM)	22·89 ± 3·88††	5	0·24 ± 0·06	4	0·11 ± 0·03
Theophylline + noradrenaline + prazosin (10 μM)	21·04 ± 3·3†	5	0·26 ± 0·05*	4	0·10 ± 0·03
Theophylline + clonidine (10 μM)	23·87 ± 2·06†††	20	0·31 ± 0·03***	23	0·15 ± 0·02†††
Theophylline + clonidine + yohimbine (1 μM)	14·40 ± 2·19††	10	0·29 ± 0·03**	7	0·07 ± 0·01‡‡

The steady-state retention of mannitol within the tissue was determined from extracts after serosal–mucosal mannitol flux had reached a steady state. The serosal permeability is estimated as described in Methods.

* Test *vs.* control, † test *vs.* theophyline, ‡ test *vs.* clonidine. One symbol $P < 0.05$; two symbols $P < 0.01$; three symbols $P < 0.001$. Significance tested with student's t test. n = number of individual determinations.

Both noradrenaline (10 μM) and clonidine (1 μM) prevented the theophylline-dependent decrease in serosal permeability to mannitol. However, following exposure to theophylline neither clonidine nor noradrenaline had any significant effect on the fractional exit rate of mannitol across the mucosal surface.

When control tissue was exposed to clonidine or noradrenaline there was no significant change in serosal permeability, but there was a clonidine-dependent increase in mucosal permeability to mannitol.

These results indicate: (a) that adrenergic agonists increased the hydraulic conductance of the submucosal tissues after exposure to theophylline or carbamyl choline; (b) neither clonidine nor noradrenaline reduced the theophylline-dependent

increase in mannitol exit via the mucosal shunt channels, probably because they also reduced the tone of the muscularis mucosa which normally holds the mucosa together.

The other results in Table 2 show that neither the β-adrenergic antagonist propanolol nor the α_1-antagonist prazosin had any significant effect on the noradrenaline-dependent increase in the steady-state retention volume of mannitol in theophylline-treated tissue. However, the effect of clonidine on serosal permeability was completely reversed by the α_2-antagonist yohimbine (1 μM). These findings are entirely consistent with the previous finding of Chang *et al.* (1982) that the augmentation of intestinal absorption is a specific α_2-adrenergic effect.

TABLE 3. Effect of dopamine on steady-state retention of [^{3}H]mannitol and fractional exit and serosal permeability of mannitol across the mucosal surface

Condition	Steady-state retention (μl cm^{-2})	n	Fractional mucosal exit (h^{-1})	n	Serosal permeability (h^{-1})
Control	24·96 ± 0·92	67	0·18 ± 0·01	70	0·09 ± 0·01
Dopamine (10 μM)	23·57 ± 1·65	5	0·25 ± 0·02**	10	0·12 ± 0·01
Theophylline (10 mM)	14·77 ± 0·93***	48	0·29 ± 0·02***	51	0·07 ± 0·01*
Dopamine (10 μM) + theophylline (10 mM)	22·53 ± 1·49†††	15	0·21 ± 0·02†	20	0·09 ± 0·01†

* Test *vs.* control; † test *vs.* theophylline. One symbol $P < 0·05$, two symbols $P < 0·01$, three symbols $P < 0·001$.

Effects of dopamine on steady-state retention volume of mannitol and the fractional exit of mannitol across the mucosal surface

The effect of dopamine (10 μM) on steady-state retention volume and fractional mucosal exit, and the estimated value of serosal permeability to mannitol in control and theophylline-treated tissue is shown in Table 3. Both dopamine and theophylline separately caused significant increases in fractional mucosal exit of mannitol (FE). However, when present together, there was no significant enhancement of mucosal exit. This indicates that dopamine and theophylline are increasing the mucosal exit of mannitol by different processes. Dopamine reverses the theophylline-dependent decrease in serosal permeability to mannitol.

Effects of theophylline and clonidine on transepithelial PD and electrical resistance

During the initial period that the tissue was exposed to bathing solution there was usually a decrease in tissue electrical resistance with consequent decrease in transepithelial PD. This period of adaptation usually lasted for about 15–20 min. The effects of theophylline on transepithelial PD and resistance are shown in Fig. 5*A* and *B*. Theophylline was added after the PD had stabilized. Following exposure to theophylline, transepithelial PD increased rapidly ($t_{\frac{1}{2}} \approx 2$ min). The transepithelial resistance also increased (Fig. 5*B*), but more slowly than PD ($(t_{\frac{1}{2}} \approx 15$ min). These records are more accurate than previous ones (Holman, Naftalin, Simmons & Walker, 1979), because of the superior design and accuracy of the present monitoring

TABLE 4. Derived values of submucosal hydraulic conductance, L_p, distensibility, K, and interstitial pressure, P_i, from the change in transerosal flow induced per mosmole

Condition	J_s (μl cm^{-2} h^{-1})	L_p ($\times 10^8$ cm s^{-1} cmH$_2$O^{-1})	K ($\times 10^3$ cmH$_2$O^{-1})	P_i (cmH$_2$O)
Control	8·6±0·6	5·7±1·0	2·4±0·5	42±6
Theophylline (10 mM)	5·9±0·3**	3·7±0·5*	2·1±0·3	45±5
Clonidine (1 μM)	7·9±0·5	6·6±0·7	2·9±0·4	33±5
Clonidine (1 μM)+ theophylline	6·9±0·3*	6·2±0·5††	3·1±0·3†	31±2†
Clonidine+ yohimbine (1 μM)+ theophylline	6·0±0·2	4·0±0·2**	2·4±0·2	42±2

* Test *vs.* control; † test *vs.* theophylline. One symbol $P < 0{\cdot}05$, two symbols $P < 0{\cdot}01$.

Value of control $L_p = 5{\cdot}7 \times 10^{-8}$ cm s^{-1} cmH$_2$O^{-1} and control $K = 2{\cdot}4 \times 10^{-3}$ cmH$_2$O^{-1}.

The values of K are estimated in all tissues by assuming that the interstitial space is initially distended by 10% above the resting level. This assumption is valid providing no large-scale changes in tissue volume occur.

TABLE 5. Effects of theophylline and clonidine on rabbit ileal PD short-circuit current (SCC) and transepithelial resistance

Condition	PD (mV)	δPD (mV)	Resistance (Ω cm^2)	δ Resistance (Ω cm^2)	SCC (μA cm^{-2})	δSCC (μA cm^{-2})
Control	1·1±0·6 (8)	—	32·3±5·4 (8)	—	35·3±5·3 (8)	—
Clonidine (5 μM) 10 min	0·6	−0·50±0·04 (8)	25·6	−6·7±0·4 (8)	25	−10·3±0·6 (8)
Theophylline (2 mM)						
Initial	2·8	+1·5±0·1 (8)	—	0	82·0	+46·7±3·7 (8)
30 min	2·8	—	40·3	+8·0±2·7 (8)	53·1	+17·8±5·7 (8)
Theophylline+ 5 min clonidine	1·5	−1·1±0·1 (6)	29·3	−10·9±1·3 (6)	51·7	−1·5±0·8 (n.s.) (6)

The times assigned to the conditions refer to the periods of exposure to the condition prior to monitoring the observed changes. n.s., not significant. Numbers in parentheses indicate number of independent estimates (n).

equipment. When clonidine (5 μM) was added following a period of exposure to theophylline sufficient to cause a significant increase in resistance, there was a decrease in transepithelial PD and a simultaneous decrease in resistance (Fig. 5*A* and *B*).

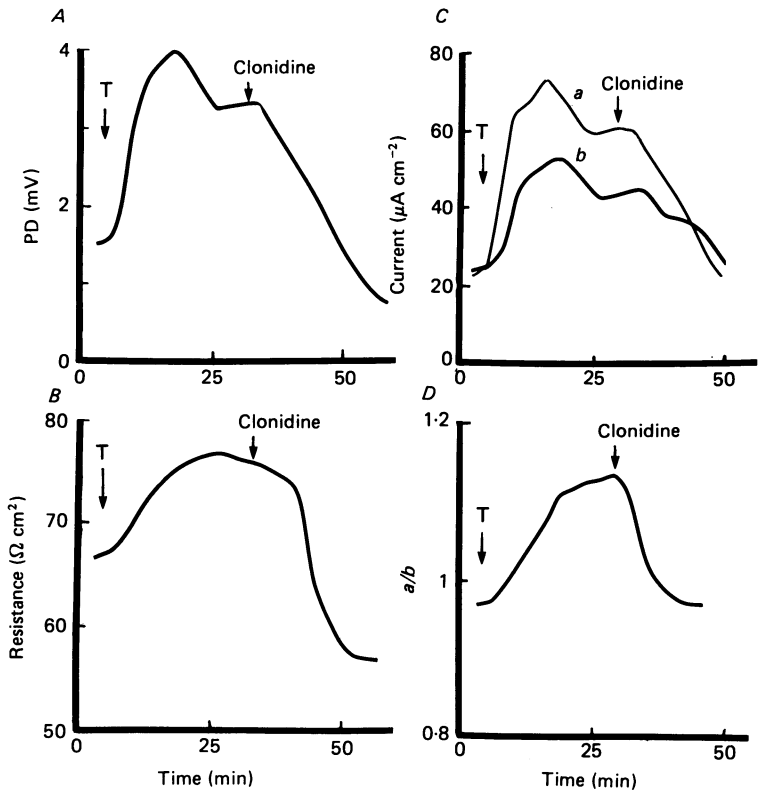

Fig. 5. Effect of theophylline, T (2 mM), on transepithelial PD (mV) and resistance (Ω cm^2); exposed tissue area 1·79 cm^{-2}. The traces shown in panels *A*, *B* and *C* are the changes in PD, resistance and short-circuit current respectively. Clonidine (5 μM) reverses the theophylline-dependent increase in PD and in resistance. The times of addition of theophylline and clonidine are indicated by the arrows. In panel *C* is shown the short-circuit current trace uncorrected for resistance change (line *a*), or corrected for tissue resistance change (line *b*). In panel *D* is shown the ratio of line *a*: line *b* from panel *C*.

This rapid increase in PD and the slower increase in the resistance indicate that two separate processes were initiated by theophylline within the tissue. The correlation of the fall in resistance and PD after exposure to clonidine suggests that clonidine may affect only the slower process.

In Table 5 the effects of theophylline and clonidine, applied both separately and together, on PD resistance and short-circuit current changes are shown. Within 5 min of application of clonidine (5 μM) to control tissue there was a significant decrease in PD, resistance and short-circuit current ($P < 0{\cdot}01$ for all effects).

Although clonidine reduced the short-circuit current in control tissue, it had no

significant effect on short-circuit current in tissues previously exposed to theophylline. It is uncertain whether the effects of clonidine on short-circuit current and PD are also not a consequence of the change in resistance.

DISCUSSION

Four aspects to the experimental results require discussion: the relationship of submucosal tone, or distensibility, to fluid movements across the serosal and mucosal surfaces; the relationship between changes in electrical PD, electrical resistance and transepithelial fluid and mannitol movements; the mode of action of catecholamines on submucosal and mucosal flows and the relationship of the effects of catecholamines *in vitro* to the effects on intestinal transport *in vivo*.

The relationship between interstitial pressure, distensibility and transepithelial fluid movements

Submucosal or transerosal flows. The data on the hydraulic and mannitol conductance of rabbit ileum in this and previous papers (Naftalin & Tripathi, 1985, 1986; Ahsan *et al.* 1987) indicate that there is a good correlation between mucosal and serosal hydraulic and mannitol permeabilities, suggesting that water and mannitol are likely to share major common pathways across both surfaces.

The hydraulic conductance of the mucosal surface of rabbit ileum is 3- to 6-fold higher than serosal surface. The mannitol permeability of the mucosal surface is between 2- and 4-fold higher than that of the serosal surface. Theophylline reduces serosal hydraulic conductance by 35% and mannitol permeability by 22%. Clonidine increases the serosal hydraulic conductance of theophylline-treated tissues by 78% and the serosal mannitol permeability of theophylline-treated tissue by 104% (Tables 1, 2 and 3).

Effect of theophylline on submucosal hydraulic and mannitol permeability. The following results indicate that theophylline decreased the hydraulic conductance of the submucosal tissues and that α_2-adrenergic agonists increased the permeability of the submucosa which has been previously reduced by theophylline. (a) The submucosal L_p was decreased by theophylline (Table 1) and was returned to control levels by clonidine. This effect was prevented by the α_2-antagonist yohimbine. (b) The electrical resistance of the tissue was raised by theophylline. This effect was reversed by clonidine (Fig. 5, Table 5). (c) The estimated permeability of the serosal surface to mannitol was decreased by theophylline and increased by clonidine and dopamine (Tables 2 and 3).

Interstitial pressure. In control tissues, the serosal L_p was $5{\cdot}7 \times 10^{-8}$ cm s^{-1} cmH_2O^{-1} (Table 1) and the interstitial pressure required to generate a flow across the serosal surface of 8·6 μl cm^{-2} h^{-1} (Fig. 1) was approximately 42 cmH_2O. The L_p in theophylline-treated tissue was $3{\cdot}7 \times 10^{-8}$ cm s^{-1} cmH_2O^{-1} (Tables 1 and 4). Hence, the pressure required to generate the observed flow of 6·0 μl cm^{-2} h^{-1} (Fig. 1) was 45 cmH_2O. Following addition of clonidine to theophylline-treated tissue, the serosal L_p increased from 3·7 to $6{\cdot}2 \times 10^{-8}$ cm s^{-1} cmH_2O^{-1} (Tables 1 and 4), but serosal outflow increased only from 6·0 to 7·1 μl cm^{-2} h^{-1} (Fig. 1). This small increase in J_s relative to the increase in serosal L_p indicates that clonidine also induced a decrease in interstitial pressure in tissue exposed to theophylline (from 45 to 31 cmH_2O).

Distensibility. The large apparent decrease in interstitial pressure following exposure of theophylline-treated tissue to clonidine can be explained on the basis that clonidine increases the tissue distensibility. The values of K, along with estimates of P_i, are shown in Table 4.

Following addition of clonidine to tissue exposed to theophylline, there is an increase in the distensibility. However, neither theophylline nor clonidine alone change the distensibility of control tissue significantly. This result may reflect the fact that distensibility is determined indirectly; this interpretation depends on the assumption that the fractional distension of the tissue is unchanged during the relatively short time of estimation of serosal L_p. These data nevertheless suggest that clonidine reduces the submucosal 'tone' and hence fluid and solute outflow across the serosal border are not increased as much as would be expected on the basis of clonidine-induced changes in L_p.

Previously, Lee (1983*b*) has also observed a correlation between fluid secretion and tone in rat intestine *in vivo*. However, measurements by insertion of micropipettes into villous lymphatics indicate interstitial pressures much lower than those required to induce flow across the serosal surface of rabbit ileum (Lee, 1986). The low pressures observed by direct insertion of micropipettes into the tissue may in part be caused by local tissue damage resulting from insertion of the micropipette. This could also explain why there is an apparent difference in hydraulic pressure between lymphatics and interstitium.

Mucosal flows. The following experimental evidence indicates that there is enhanced reflux of fluid and solute across the mucosal surface after exposure to theophylline: (i) the fractional exit of mannitol across the mucosal surface was raised following exposure to theophylline (Tables 2 and 3); (ii) following exposure to theophylline (Fig. 1) J_m changed simultaneously with the changes in J_s.

If the L_p of the mucosal shunt pathway is $\approx 3 \times 10^{-7}$ cm s^{-1} cmH_2O^{-1} (Naftalin & Tripathi, 1985, 1986) then a theophylline-dependent increase of interstitial pressure will increase fluid reflux via the paracellular shunt pathway and so reduce net fluid uptake across the mucosal surface (Fig. 1).

The clonidine-dependent increase in distensibility is sufficient to reduce interstitial pressure from 45 to 32 cmH_2O (Table 4). This would increase net mucosal inflow by reducing fluid reflux via the shunt channels by 14 μl cm^{-2} h^{-1}. However, the observed clonidine-dependent increase is only 7 μl cm^{-2} h^{-1} (Fig. 1). A possible reason for this shortfall is that clonidine also increases the mucosal shunt conductance (Table 2). Dopamine raised the mucosal mannitol permeability (Table 3). However, dopamine also reduced the theophylline-dependent increase in mannitol permeability.

These data suggest that raised backflux of solute across the mucosal shunt can arise from (a) increased interstitial pressure caused by increased submucosal tone and (b) increased shunt conductance, perhaps due to relaxation of the contractile elements within the mucosa, e.g. the muscularis mucosa.

The relationship between changes in transepithelial electrical PD, electrical resistance and transepithelial fluid movements

As stated in the Introduction, the effects of theophylline and catecholamines on transepithelial PD suggest a direct action on enterocyte fluid and ion movements. The results showing agonist binding to receptors on the enterocyte membranes, or

effects on enterocyte cyclic nucleotide metabolism, do not unambiguously indicate that the mucosal cells are the site of control of tissue fluid and electrolyte movement since alternative binding sites are present within the tissue. Moreover, even the electrical changes (Fig. 5 and Table 5) cannot be ascribed solely to mucosal processes. Following exposure to theophylline, the transepithelial PD rose by approximately 2 mV within 5 min. The average tissue resistance after a short delay rose slowly from 25 to 40 Ω cm^2 (Table 5) within 30 min, and within an hour to 65 Ω cm^2 ($t_{\frac{1}{2}} \approx 15$ min). When clonidine was applied to theophylline-treated tissue after an increase in resistance had occurred, the transepithelial PD decreased by 1·1 mV and resistance decreased by 10·9 Ω cm^2 simultaneously, without any significant change in short-circuit current. This indicates that clonidine affects the process causing the slow resistance change, rather than the process affecting the rapid PD change. This effect is illustrated in Fig. 5 (panels *C* and *D*), where it is seen that clonidine causes a rapid decrease in short-circuit current when estimated without correcting for the theophylline-induced increase in tissue resistance, but causes a much smaller decrease in short-circuit current when this correction is applied.

The most likely cause of the slow theophylline-dependent increase in resistance is the decrease in interstitial hydration and consequent increase in tortuosity of the submucosal matrix (Holman *et al.* 1979). This explanation for the resistance change resulting from exposure to α-agonists may also explain why catecholamine-dependent changes in transepithelial electrical potential are sometimes absent, as the changes in resistance will depend on the previous condition of the tissue (Field & McColl (1973) and Hubel (1976)).

The mode of action of catecholamines on submucosal and mucosal flows

The data in Figs 2, 3 and 4 indicate that there are separate α_2-adrenoreceptors and specific dopaminergic receptors in rabbit ileum. Stimulation of both types of receptors enhanced absorption following an antiabsorptive stimulus. Both types of agonist act by inducing a relaxation of the submucosal tissues, hence increasing the hydraulic conductivity and reducing interstitial pressure. The mode of action of the catecholamines on the submucosal tissues is likely to be complex. Direct effects on smooth muscle are possible, but inhibition of acetylcholine release by stimulation of dopamine and α_2-adrenoreceptors on the presynaptic membranes as suggested by the studies on inhibition of acetylcholine release is also possible (Paton & Vizi, 1969; Vizi *et al.* 1974; Drew, 1978; Kilbinger & Wessler, 1979; Gorich *et al.* 1982).

Alternative interpretations. A conventional interpretation of some of the results here, and also those in Ahsan *et al.* (1987), would be that inhibition of fluid absorption by secretagogues is attributable solely to changes in mucosal processes. The latter could be a consequence of an inhibition of electroneutral NaCl across the mucosal border, which is thought to occur in addition to the activation of Cl^- secretion across the apical membrane, possibly from crypt cells (Frizzell, Field & Schultz, 1979).

Hence, the antisecretory effect of catecholamines and of opiates (Ahsan *et al.* 1987), without reversal of the effect of secretagogues on short-circuit current and transepithelial PD, could be ascribed simply to the recovery of the electroneutral NaCl influx without concurrent reversal of Cl^- secretion. The main reason for rejection of this hypothesis is that Cl^- exchange across the mucosal surface of tissues

exposed to theophylline and choleragen is increased, rather than decreased, as would be expected if there were a significant decrease in mucosal NaCl influx (Naftalin & Simmons, 1979). This result was confirmed by direct estimates of increased Cl^- conductance in *Necturus* gall-bladder epithelium exposed to cyclic AMP (Petersen & Reuss, 1983). A way to reconcile these data with the observed decrease in mucosal–serosal Cl^- flux, and decreased net uptake of Cl^- into mucosa exposed to secretagogues, is that secretagogues in addition to increasing Cl^- exchange and conductance across the mucosal surface, also enhance reflux of NaCl via shunt channels. This enhanced reflux via shunt channels is demonstrated in this paper by direct methods. It remains likely that both mucosal and submucosal processes are activated during fluid secretion.

The relationship between intestinal fluid transport in vitro to in vivo

Catecholamines increase intestinal fluid and electrolyte absorption both *in vivo* (Hubel, 1976; Sjövall *et al.* 1983; Lee, 1983*b*; Wahawisan *et al.* 1985) and *in vitro* (Field & McColl, 1973; Donowitz *et al.* 1982). It could be argued that the results in this paper are only applicable to the *in vitro* situation since interstitial pressure and hence mucosal backflux are lower *in vivo* than *in vitro*. The estimates of steady-state interstitial pressure *in vitro* are larger than those obtained from stopped flow pressures in rat intestinal lymphatics *in vivo* (approximately 5 cmH_2O) (Lee, 1983*a*). Whilst a relationship between tone and fluid absorption has been observed *in vivo* (Lee, 1983*b*) this may depend on interactions other than those observed *in vitro*.

Further studies of the mechanical factors affecting *in vivo* fluid absorption are needed before these questions can be answered.

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